RESEARCH ARTICLE

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Multigene Panel Testing Reveals Novel Variants in Hereditary Spherocytosis Patients in Türkiye

Türkiye'deki Herediter Sferositoz Hastalarında Çoklu Gen Panel Testi ile Yeni Varyantların Saptanması

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Abstract

Objective: This study aimed to determine the genotypic characteristics of patients with hereditary spherocytosis (HS) in Türkiye and to examine the correlation between genotype and phenotype.

Materials and Methods: We analyzed the cases of 18 patients admitted to the pediatric hematology outpatient clinic with hemolytic anemia, jaundice, cholelithiasis, and splenomegaly. According to the Eber classification, the patients' clinical presentations were categorized as mild, moderate, or severe. Next-generation sequencing was used to analyze single-nucleotide and copy-number variations in all genes associated with HS via clinical exome sequencing. Relationships between the genes with detected variants and the clinical presentations of the patients were investigated.

Results: In total, 21 variants were detected in 5 HS-related genes. Twelve of them were previously reported variants and 9 were novel variants. Seven of them were pathogenic and two were classified as variants of uncertain significance according to the American College of Medical Genetics and Genomics. We discuss the phenotypic effects of novel pathogenic variants in the *SPTA1*, *SPTB*, *ANK1*, *SLC4A1*, and *EPB42* genes. Patients with pathogenic *EPB42* and *SLC4A1* variants had less severe clinical findings compared to other gene variants according to the Eber classification. On the other hand, patients with pathogenic variants of *SPTA1* and *SPTB* had more severe clinical presentation.

Conclusion: Molecular diagnosis of HS is important for treatment, prediction of the clinical outcome, and appropriate genetic counseling. Our study contributes to knowledge of the genotype-phenotype distribution of HS by introducing novel variants to the literature.

Keywords: Hereditary spherocytosis, Next-generation sequencing, Genotype-phenotype correlation

Öz

Amaç: Bu çalışmanın amacı Türkiye'deki herediter sferositoz (HS) hastalarının genotipik özelliklerini belirlemek ve genotip ile fenotip korelasyonunu incelemektir.

Gereç ve Yöntemler: Bu çalışmada, hemolitik anemi, sarılık, safra taşı ve splenomegali ile çocuk hematoloji polikliniğine başvuran 18 hasta incelenmiştir. Eber sınıflandırmasına göre hastaların klinik bulguları hafif, orta ve ağır olarak kategorize edilmiştir. HS ile ilişkili tüm genlerdeki tek nükleotid ve kopya sayısı değişikliklerini analiz etmek için klinik ekzom dizileme kiti ile yeni nesil dizileme yöntemi kullanılmıştır. Hastalarda varyant saptanan genler ile klinik prezentasyon arasında ilişki olup olmadığı araştırılmıştır.

Bulgular: HS ilişkili beş gende toplam 21 varyant tespit edilmiştir. Bunlardan 12'si tanımlı varyantlar, dokuzu ise yeni varyantlardır. Yedi tanesi patojenik olup ikisi Amerikan Klinik Genetik ve Genomik Koleji'ne göre klinik önemi bilinmeyen varyant olarak sınıflandırılmıştır. Bu çalışmada, *SPTB, ANK1*, *SLC4A1*, *SPTA1* ve *EPB42* genlerindeki yeni patojenik varyantların fenotipik etkileri tartışılmıştır. Eber sınıflamasına göre, *EPB42* ve *SLC4A1* genlerinde patojenik varyantları olan hastalar diğer gen varyantlarına kıyasla daha hafif klinik bulgular göstermiştir. Öte yandan, *SPTA1* ve *SPTB* genlerinin patojenik varyantlarını taşıyan hastalar daha ciddi klinik seyre sahip olmuştur.

Sonuç: HS'nin moleküler tanısı, tedavi, klinik sonucun öngörülmesi ve uygun genetik danışmanlık için önemlidir. Sonuç olarak, çalışmamız literatüre yeni varyantlar kazandırarak HS'nin genotip-fenotip dağılımına katkı sağlayacaktır.

Anahtar Sözcükler: Herediter sferositoz, Yeni nesil dizileme, Genotip-fenotip korelasyonu

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Introduction

Hereditary anemias (HAs) constitute a highly diverse group. Pathogenic variants in more than 70 genes cause HAs [1]. The most common cause of hereditary hemolytic anemia is hereditary spherocytosis (HS) [1]. HS is a disease that exhibits heterogeneity both clinically and genetically. The common clinical manifestations are hemolytic anemia, splenomegaly, jaundice, and cholelithiasis. The wide clinical spectrum of HS ranges from very mild disease to very severe cases requiring splenectomy and transfusions. Although studies on genotype-phenotype correlations in HS have been conducted over the past decade, clear correlations have yet to be established [2].

As a result of pathogenic variants in genes encoding membrane or cytoskeleton proteins, spherical-shaped red blood cells are formed [3]. To date, 5 genes (*ANK1* [ankyrin; OMIM: 612641], *SPTA1* [a-spectrin; OMIM: 182860], *SPTB* [b-spectrin; OMIM: 182870], *EPB42* [protein 4.2; OMIM: 177070], and *SLC4A1* [band-3; OMIM: 109270]) that cause HS have been identified [4,5,6,7,8]. *SPTA1* and *EPB42* are related to autosomal recessive (AR) inheritance, *SPTB* and *SLC4A1* cause autosomal dominant (AD) inheritance, and *ANK1* is responsible for both AR and AD inheritance. Overall, AD and AR inheritance patterns are present in 75% and 25% of patients, respectively. Pathogenic variants are most commonly detected in the *ANK1* gene (40%-65%) in patients with HS, followed by *SLC4A1* (20%-35%) and *SPTB* (15%-30%), respectively [9].

Due to non-specific and overlapping clinical findings, the differential diagnosis and classification of HAs is not easy. Genetic testing may be necessary for these patients to confirm the clinical diagnosis, provide appropriate genetic counseling to the family, establish genotype-phenotype correlations based on the accumulated literature, and allow for accurate assessment of the clinical severity and proper monitoring of the case.

This study aimed to determine the genotypic characteristics of HS patients from among our cases with clinical exome sequencing and to examine the correlations between phenotypes and genotypes.

Materials and Methods

Patients

From May 2020 to September 2023, patients who had a clinical diagnosis of HS with the presence of spherocytes on peripheral smear, were admitted with non-immune hemolytic anemia, and had negative tests for hemoglobinopathies, pyruvate kinase, and glucose-6-phosphate dehydrogenase deficiency were evaluated with a next-generation sequencing (NGS) multigene panel. Osmotic fragility testing was performed for all patients and an increase in osmotic fragility was observed in all cases. Our cohort included 18 unrelated probands in Türkiye with HS-associated pathogenic variants. The epidemiological, clinical, and laboratory results were evaluated retrospectively.

The classification of disease severity was based on hemoglobin, reticulocyte percentage, and serum total bilirubin levels, as in the Eber classification [2,3]. Based on the Eber classification, the clinical presentation of HS was categorized as mild, moderate, or severe. In the mild form, hemoglobin levels are in the range of 11-15 g/dL, reticulocyte counts are 3%-6%, and bilirubin levels are 17-34 µmol/L, with splenectomy typically not required. The moderate form is characterized by hemoglobin levels of 8-12 g/dL, reticulocyte counts exceeding 6%, and bilirubin levels above 34 µmol/L, with splenectomy often being necessary before puberty. In severe HS, hemoglobin levels fall between 6 and 8 g/dL, reticulocyte counts exceed 10%, and bilirubin levels are above 51 µmol/L, with splenectomy being almost universally indicated. According to the severity of hemolysis, 7 of our patients were in the mild group, 6 were in the moderate group, and 5 were in the severe group. Among the 5 patients in the severe group, two had undergone splenectomy. The Local Ethics Committee of the Marmara University Faculty of Medicine approved the study (approval number: 1770, date: 08.12.2023).

Molecular Studies

Patients' peripheral blood DNA was isolated using the QIAamp DNA Mini Kit (QIAGEN, Germantown, MD, USA). Quantification of the extracted DNA was performed using a Qubit 3.0 Fluorometer (Thermo Fisher Scientific, Waltham, MA, USA). DNA with absorption ratios between 1.8 and 2.0 for the 260/280 nm ratio and between 1.6 and 2.4 for the 260/230 nm ratio, with a concentration ranging from 50 to 100 ng/µL, was used. Briefly, 1 µg of genomic DNA was fragmented using an ultrasonication device (Covaris, Woburn, MA, USA). The resulting fragments were end-repaired, adapter-ligated, and subjected to size selection before being enriched. Sequencing was performed via the NextSeq 550 platform (Illumina, San Diego, CA, USA). Approximately 10 GB of sequence data was generated for each sample by loading it onto a flow cell lane, producing 2x100-bp paired-end reads. The reads were mapped to the human genome reference build GRCh37/hg19 using the Burrows-Wheeler aligner algorithm. Polymerase chain reaction duplicates were marked with GATK v1.64 and the output was converted to BAM format. The mean exome coverage was 66x, with 96.5% of target bases covered by more than 10x and 83.8% covered by more than 30x. The Sophia DDM-V4 platform (Sophia Genetics, Boston, MA, USA) was used for data analysis.

Single-nucleotide variants and copy-number variants of *SPTB* (NM_000347), *SPTA1* (NM_003126), *ANK1* (NM_000037), *EPB42* (NM_000119), and *SLC4A1* (NM_000342) were evaluated. The Clinical Exome Solution v3 panel (SOPHiA Genetics, USA) was utilized; it targets 5,800 genes associated with inherited diseases. Among these genes are those associated with congenital

dyserythropoietic anemias, Diamond-Blackfan anemia, hereditary stomatocytosis, glucose-6-phosphate dehydrogenase deficiency, and pyruvate kinase deficiency. These genes were analyzed for each patient in our cohort and any patients with variants in these genes were not included in the study. Rare variants were searched in ClinVar (http://www.ncbi.nlm.nih.gov/clinvar) and the Human Gene Mutation Database (http://www.hgmd.cf.ac.uk) to determine whether they had been reported before. Variants were also searched in the current literature, and if they had been previously described in the literature they were not considered novel. Pathogenicity of retained novel variants was determined following the criteria of the American College of Medical Genetics and Genomics (ACMG) [10]. Segregation was analyzed using the MiSeq platform (Illumina, USA).

Results

The laboratory and clinical findings of 18 patients in Türkiye who were found to have variants related to HS are presented in Table 1. Eleven of the patients were male (61%) and the median age was 8 years, with ages ranging from 2 to 58 years. Only six patients had elevated mean corpuscular hemoglobin concentration (MCHC) levels.

Nine patients had *SPTB*, 3 patients had *SPTA1*, 3 had *ANK1*, 2 had *SLC4A1*, and 2 had *EPB42* variants. We detected a total of 21 variants in these 18 patients because one patient had both *SPTA1* and *SPTB* variants (patient 14). Of the patients with *SPTB* variants, 2 cases were evaluated as mild, 3 as moderate, and 3 as severe HS. Among the 2 patients who had *SPTA1* variants (patients 13 and 18), one had severe and the other had moderate HS. Three patients had *ANK1* variants (patients 5, 10, and 15), with one case being moderate HS and the others being severe HS. Among the 2 patients with *SLC4A1* variants (patients 1 and 8), one was classified as having mild and the other moderate HS. The 2 patients with *EPB42* variants (patients 6 and 17) displayed mild clinical findings. The patient who had both *SPTA1* and *SPTB* variants (patient 14) was classified as having mild HS.

In total, 21 variants were detected in the 5 HS-related genes. Twelve of them were previously reported variants and 9 of them were novel variants (Table 2; Figure 1). Of the detected variants, 10 caused premature protein termination, while 11 were non-truncating variants (Figure 2). A total of 9 variants were identified in the *SPTB* gene; thus, this gene had the highest frequency of detected variants. The numbers of detected variants in the *SPTA1*, *SLC4A1*, *ANK1*, and *EPB42* genes were 3, 2, 3, and 2, respectively.

In our cohort, 9 novel variants were detected. By the ACMG criteria, 7 of them were pathogenic and 2 were variants of unknown significance (VUSs). Four novel pathogenic variants were identified in the *SPTB* gene, 2 in the *SLC4A1* gene, 2 in *SPTA1* gene, and 1 in the *ANK1* gene. Of the novel variants in the *SPTB* gene, 2 were nonsense variants (c.5587C>T, p.Gln1863*; c.3450G>A, p.Trp1150*; patients 3 and 12, respectively), 1 was

Table 1. Clinical and laboratory findings of patients with hereditary spherocytosis.

ID	Sex	Age	Severity	Hb	Ret	Bilirubin	MCHC	Splenomegaly on USG	Cholelithiasis	Transfusion	Splenectomy
Patient 1	M	58	Mild	12.7	7.2	2.0	37.3	No	No	No	No
Patient 2	M	8	Severe	6.9	5.1	4.6	34.1	Yes	Yes	Yes	Yes
Patient 3	M	4	Moderate	8.2	11.0	3.3	33.6	Yes	Yes	No	No
Patient 4	M	8	Severe	6.7	10.8	7.8	34.2	Yes	Yes	Yes	Yes
Patient 5	M	3	Moderate	9.9	6.0	0.2	34.0	Yes	Yes	No	No
Patient 6	F	5	Mild	11.7	4.4	3.2	34.7	No	No	No	No
Patient 7	F	9	Moderate	8.8	14.5	3.4	34.1	Yes	Yes	Yes	No
Patient 8	F	7	Moderate	9.9	9.9	3.4	36.1	Yes	Yes	No	No
Patient 9	F	33	Moderate	10.1	8.6	2.8	34.9	No	No	No	No
Patient 10	M	3	Severe	6.8	9.8	3.0	31.9	Yes	Yes	Yes	No
Patient 11	M	10	Mild	12.9	0.9	2.3	33.9	Yes	Yes	No	No
Patient 12	F	15	Mild	13.3	3.2	0.8	37.0	No	No	No	No
Patient 13	M	8	Moderate	8.9	6.7	6.9	32.4	Yes	Yes	Yes	No
Patient 14	M	19	Mild	13.2	1.2	2.1	35.7	Yes	Yes	No	No
Patient 15	M	4	Severe	7.0	16.3	1.9	35.5	Yes	Yes	Yes	No
Patient 16	M	44	Mild	13.5	3.4	0.6	33.2	Yes	NA	No	No
Patient 17	F	2	Mild	11.3	4.4	1.0	34.5	Yes	Yes	No	No
Patient 18	F	6	Severe	7.1	4.6	1.7	39.0	Yes	Yes	Yes	No

M: Male; F: female; Hb: hemoglobin; Ret: reticulocytes; MCHC: mean corpuscular hemoglobin concentration; USG: ultrasonography. Hb (g/dL) reference values: 11.5-15.0; Ret % reference value: 0.5-2; MCHC (g/dL) reference values: 31.6-35.4. For patients with splenectomy, Hb levels before splenectomy are presented.

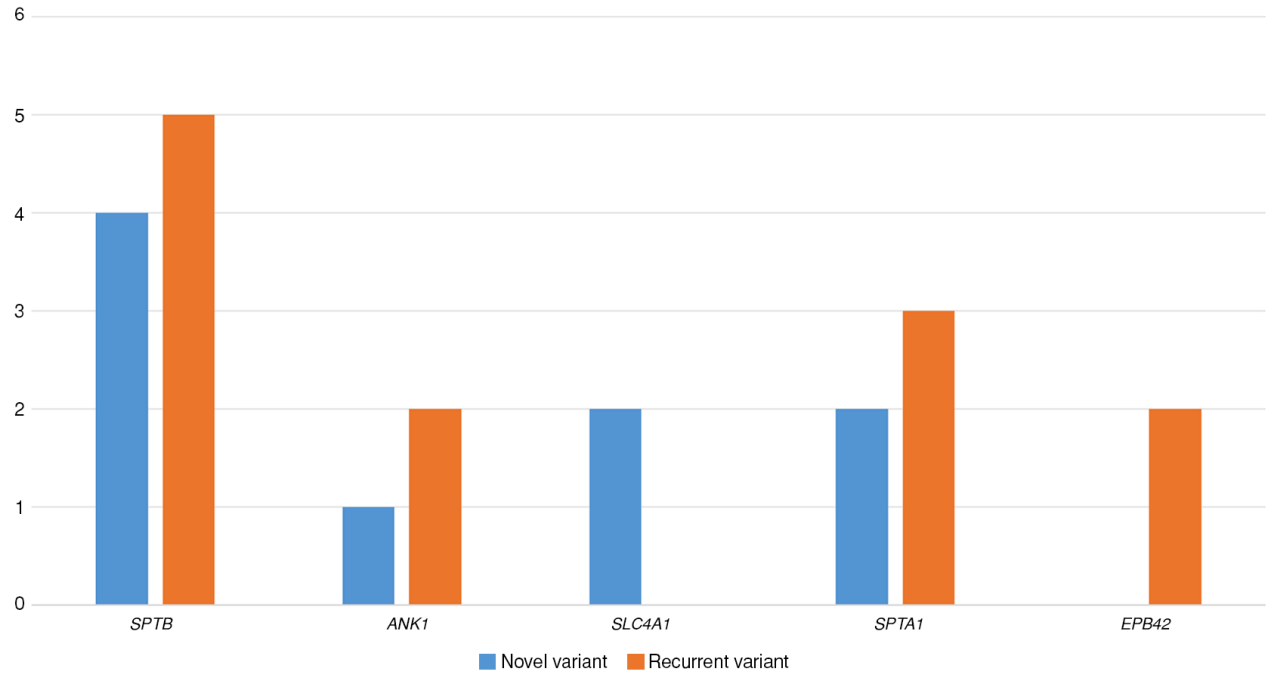

Figure 1. Presentation of previously reported and novel variants in 5 genes associated with HS.
HS: Hereditary spherocytosis.

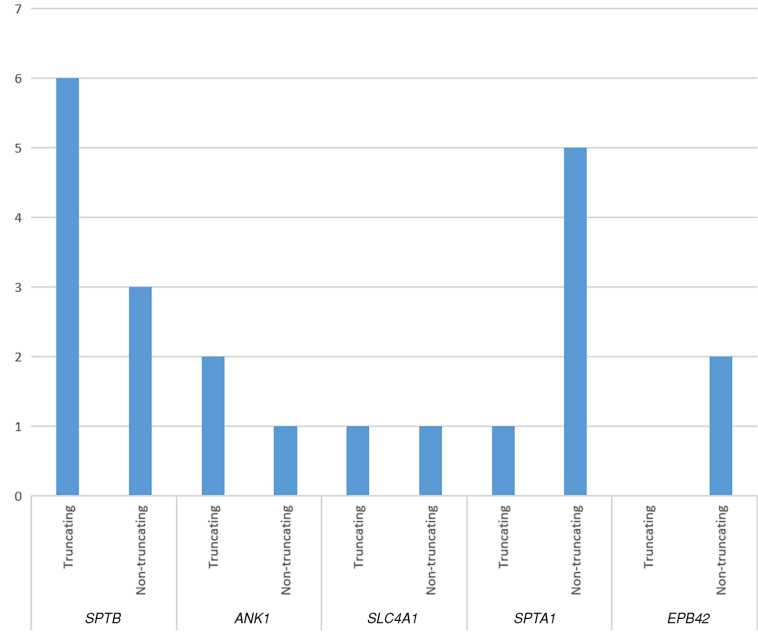

Figure 2. Representation of truncating and non-truncating variants identified in genes associated with hereditary spherocytosis.

a splice-site variant (c.3561+1G>C; patient 9), and 1 was a frameshift variant (c.4832dupA, p. Ile1612Aspfs*5; patient 2). The novel variant in the *ANK1* gene was a frameshift variant (c.1771dupC, p.Arg591Profs*30; patient 15). One of the 2 novel variants identified in the *SLC4A1* gene was a missense variant (c.2422C>A, p.Arg808Ser; patient 8) and the other was a splice-site variant (c.1801-2A>C; patient 1). The novel pathogenic variant identified in the *SPTA1* gene in this study was the sole gross deletion detected, which encompassed exons 2-52 (patient 13). Three of the novel VUSs were missense variants and 1 was a synonym variant. Two of them (c.6026G>A, p.Arg2009His; c.3208C>A p.Arg1070Arg) were identified in the *SPTA1* gene, 1 (c.1195G>A, p.Ala399Thr) was found in the *ANK1* gene, and 1 (c.1370G>A, p.Arg457His) was found in the *EPB42* gene, affecting patients 14, 13, 5, and 6, respectively. All detected variants are shown in Figure 3.

Novel Pathogenic Variants and Phenotypic Effects

The c.3450G>A variant causes a transition from guanine to adenine at position 3450 of exon 15 in the *SPTB* gene (patient 12). This change leads to the 1150th codon becoming a stop codon, causing premature termination of the protein. This variant, which was not reported in ClinVar, was assessed as "likely pathogenic" (LP) (PVS1: null variant in a gene where loss of function is a known mechanism of disease; PM2: extremely low frequency in gnomAD population databases) according to the ACMG criteria. The patient was a 15-year-old girl who had mild disease with a slightly increased reticulocyte percentage, normal hemoglobin levels, and no splenomegaly or hyperbilirubinemia

The c.3561+1G>C variant is located at the splice donor site of exon 15 in the *SPTB* gene (patient 9). A change from guanine to cytosine, which this variant causes, occurs in the consensus donor site sequence that is typically "GT." This change is recognized as affecting gene splicing, leading to abnormal protein production. According to the ACMG criteria, this variant was evaluated as LP (PVS1, PM2). The patient with this variant was a 33-year-old woman with moderate HS.

The duplication of adenine at position 4832 in the *SPTB* gene results in the substitution of aspartic acid instead of isoleucine, which is the 1612th amino acid, and causes premature termination of the protein after the addition of 5 amino acids (patient 2). This variant was not reported in the ClinVar database. According to the ACMG criteria, this variant was classified as LP (PVS1, PM2). The patient was an 8-year-old boy who undergone splenectomy due to transfusion-dependent severe anemia.

The duplication of cytosine at the 1771st position of the *ANK1* gene causes substitution of the 591st amino acid, arginine, to proline and leads to termination after the addition of 30 amino acids. This variant was also evaluated as LP (PVS1, PM2) in line with the ACMG criteria (patient 15). The patient was a 4-year-old boy with severe HS who had profound splenomegaly and cholelithiasis, requiring frequent transfusions.

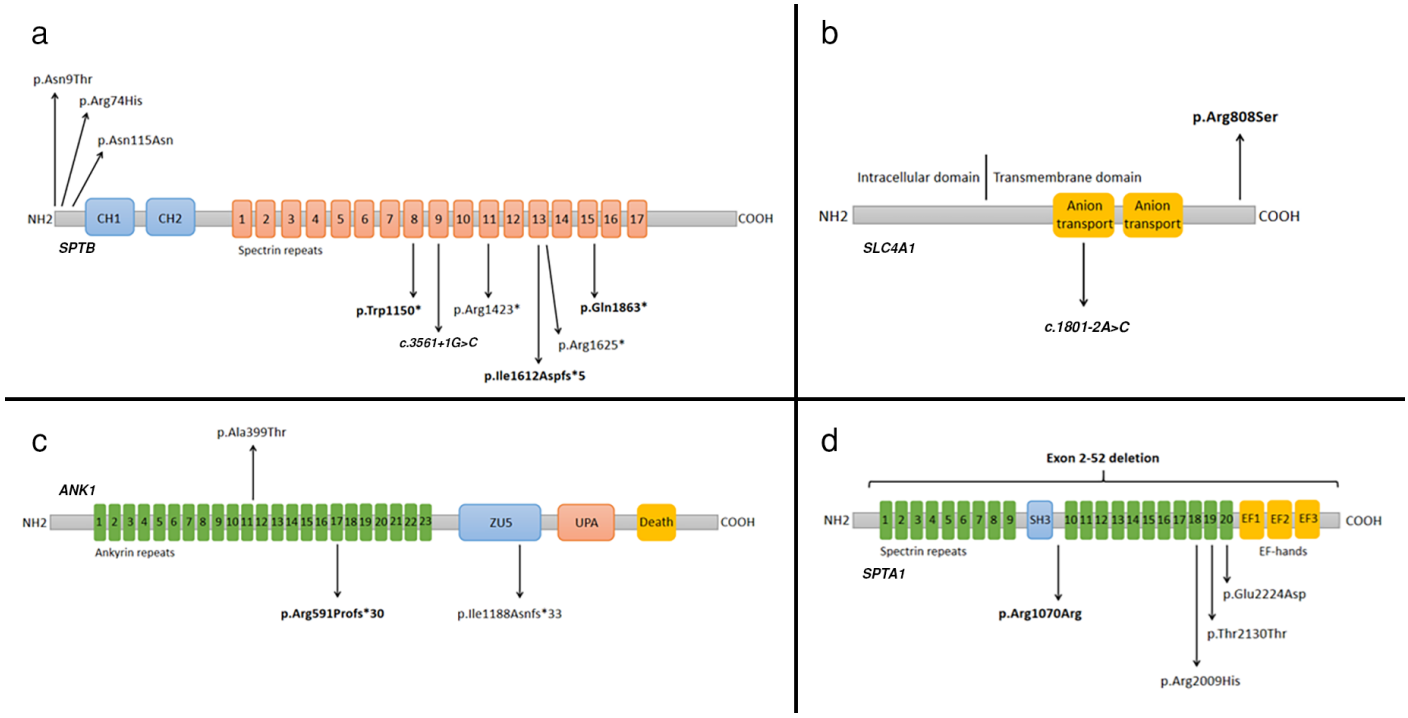

Figure 3. Schematic presentation of all variants identified in this study for the protein domains of a) *SPTB*, b) *SLC4A1*, c) *ANK1*, and d) *SPTA1*. Novel variants are shown in bold.

The substitution of cytosine with adenine at position 2422 of the *SLC4A1* gene results in a serine instead of arginine, which is the 808th amino acid. This missense variant is located at the 18th exon of the *SLC4A1* gene (patient 8). Although this variant was not reported in ClinVar, an alteration that causes different amino acid replacements at the same position (p.Arg808His) was reported as P/LP in the same database. According to the ACMG criteria, the pathogenicity of this variant was evaluated as LP (PP3: computational prediction tools unanimously support a deleterious effect on the gene; PM2, PM5: different amino acid change as a known pathogenic variant). The patient was a 7-year-old girl with moderate HS. She had splenomegaly and cholelithiasis, but transfusion was not required.

The c.1801-2A>C variant causes conversion of adenine to cytosine in the splice-acceptor site of the *SLC4A1* gene (patient 1). This change, with its effect on splicing, possibly leads to an abnormal protein product. The heterozygous c.1801-2A>C variant in *SLC4A1* was not reported in ClinVar; however, a change from A to G at the same position was described in ClinVar as LP. This supports the pathogenicity of the variant identified in our patient (PM5). The variant was assessed as LP according to the ACMG criteria (PVS1, PM2, PM5). The patient was a 58-year-old man with mild HS.

Deletion of the entire *SPTA1* gene was reported previously [11]; however, deletion of exons 2-52 of *SPTA1* has not been previously reported (patient 13). This deletion leads to a truncated protein (PVS1). Additionally, this variant is rare in population databases (PM2). According to the ACMG criteria, the pathogenicity of this variant was evaluated as LP. The patient was an 8-year-old boy who had moderate HS, splenomegaly, and cholelithiasis, necessitating intermittent erythrocyte transfusions.

Discussion

HS is the most frequent form of non-immune hemolytic anemia, exhibiting a prevalence ranging from 1/2000 to 1/5000 [1,3,10]. While most variants manifest via dominant inheritance, AR transmission is also evident in cases involving *SPTA1* and *EPB42* variants [1,7,9]. The genetic landscape of HS is influenced by ethnicity and race, contributing to diverse variations within populations. Numerous studies conducted in Europe and North America [2,9,11,12] have identified *ANK1* and *SPTB* variations as primary contributors to genetically defined HS cases, whereas in Japan [7], *EPB42* and *SLC4A1* variants were recognized as the most prevalent aberrations. However, comprehensive data on the prevalence of these variations remain limited in Türkiye.

In this study, 18 patients had 21 variants detected in *SPTB*, *SPTA1*, *EPB42*, *SLC4A1*, and *ANK1* with 9, 5, 2, 2, and 3 variants, respectively. Notably, one patient in our series presented with combined pathogenic variations in the *SPTA1* and *SPTB* genes. Spectrin variants emerged as the most frequently observed genetic alterations, accounting for 11 of 18 cases (61%) in our cohort.

Although high MCHC is typically considered a useful parameter, some studies show that it is a weak diagnostic parameter for HS [13]. Similarly, in our series, we observed elevated MCHC levels in only 6 of 18 patients (33%).

Genotype-Phenotype Correlation

HS displays significant heterogeneity. Studies focused on genotype-phenotype correlations in clinical settings are still a subject of controversy. Although the most common pathogenic variants were found in the *ANK1* gene in studies conducted in Northern Europe and the United States [9], the most common variant was found in *SPTB* in our study. Two patients had mild, 3 had moderate, and 3 had severe HS. One patient had combined variants of *SPTA1* and *SPTB*. In our series, we had 2 patients with *SPTA1* variants, one of whom had moderate HS while the other had severe HS with splenectomy. Similarly, in a study conducted in Canada, it was observed that children with pathogenic variants of *SPTA1* had the lowest hemoglobin values and were more likely to require transfusions and undergo splenectomy and cholecystectomy in childhood than patients with all other forms of HS [14]. *SLC4A1* variants are generally reported to cause mild compensated hemolytic anemia and are often diagnosed in adulthood. *SLC4A1* variants were detected in 2 of our patients. One of these patients, who had mild HS, was 58 years old, whereas the other, who had moderate HS, was 8 years old. Two studies conducted with extensive samples in Canada and the Netherlands revealed that pediatric patients exhibiting *SLC4A1* defects manifested less severe phenotypes [9,14].

Table 2. Detailed bioinformatic results of the variants identified in 18 patients in Türkiye diagnosed with HS.

Patient	Gene	cDNA	Protein	Zygosity	Variant type	ClinVar database	ACMG	Reference
Patient 1	*SLC4A1* (NM_000342)	c.1801-2A>C	-	Heterozygous	Splice-site	Not reported	LP	This study
Patient 2	*SPTB* (NM_000347)	c.4832dupA	p.Ile1612Aspfs*5	Heterozygous	Frameshift	Not reported	LP	This study
Patient 3	*SPTB* (NM_000347)	c.5587C>T	p.Gln1863*	Heterozygous	Nonsense	Not reported	LP	This study
Patient 4	*SPTB* (NM_000347)	c.345T>C	p.Asn115Asn	Heterozygous	Synonymous	VUS/Benign	LB	-

Table 2. Continued.

Patient	Gene	cDNA	Protein	Zygosity	Variant type	ClinVar database	ACMG	Reference
Patient 5	*ANK1* (NM_000037)	c.1195G>A	p.Ala399Thr	Heterozygous	Missense	VUS	VUS	-
Patient 6	*EPB42* (NM_000119)	c.1370G>A	p.Arg457His	Heterozygous	Missense	VUS	VUS	-
Patient 7	*SPTB* (NM_000347)	c.4873C>T	p.Arg1625*	Heterozygous	Nonsense	Pathogenic	P	Aggarwal et al. [13]
Patient 8	*SLC4A1* (NM_000342)	c.2422C>A	p.Arg808Ser	Heterozygous	Missense	Not reported	LP	This study
Patient 9	*SPTB* (NM_001024858)	c.3561+1G>C	-	Heterozygous	Splice-site	Not reported	LP	This study
Patient 10	*ANK1* (NM_000037)	c.3563_3564del	p.Ile1188Asnfs*33	Heterozygous	Frameshift	Likely Pathogenic	LP	-
Patient 11	*SPTB* (NM_000347)	c.4267C>T	p.Arg1423*	Heterozygous	Nonsense	Pathogenic	P	Peng et al. [15]
Patient 12	*SPTB* (NM_001024858)	c.3450G>A	p.Trp1150*	Heterozygous	Nonsense	Not reported	LP	This study
Patient 13	*SPTA1* (NM_003126)	c.3208C>A Exon 2-52	p.Arg1070Arg -	Heterozygous Heterozygous	Synonymous Deletion	Not reported Not reported	VUS -	This study This study
Patient 14	*SPTB* (NM_000347) *SPTA1* (NM_003126)	c.26A>C c.6026G>A	p.Asn9Thr p.Arg2009His	Heterozygous Heterozygous	Missense Missense	VUS VUS	VUS VUS	Russo and Andolfo [16]
Patient 15	*ANK1* (NM_000037)	c.1771dupC	p.Arg591Profs*30	Heterozygous	Frameshift	Not reported	LP	This study
Patient 16	*SPTB* (NM_000347)	c.221G>A	p.Arg74His	Heterozygous	Missense	VUS	VUS	-
Patient 17	*EPB42* (NM_000119)	c.1477G>A	p.Gly493Ser	Heterozygous	Missense	VUS	B	-
Patient 18	*SPTA1* (NM_003126)	c.6672A>C c.6390C>T	p.Glu2224Asp p.Thr2130Thr	Heterozygous Heterozygous	Missense Synonymous	LP/VUS/LB/B VUS	LB VUS	- -

ACMG: American College of Medical Genetics and Genomics; P: pathogenic; LP: likely pathogenic; VUS: variant of unknown significance; LB: likely benign; B: benign; HS: Hereditary spherocytosis.

Children with HS with a pathogenic variant of *SLC4A1* had the mildest phenotypes [9]. They also had the highest hemoglobin, lowest reticulocyte counts, and lowest unconjugated bilirubin levels compared to patients with pathogenic variants of other genes. In addition, none of them required splenectomy in childhood. Although *EPB42* variants have been reported to be very rare and usually only frequent in Japanese case series [7], 2 (11.1%) of our 18 patients had *EPB42* variants. Both had mild HS, similar to the patients previously reported in the literature [7,9]. Three of our patients had *ANK1* variants; 2 of them had severe HS while the third had moderate HS (patients 5, 10, and 15). van Vuren et al. [9] reported that pathogenic variants in the spectrin binding domains of *ANK1*, *SPTA1*, and *SPTB* cause more severe phenotypes. Although our study demonstrated that patients with *ANK1* variants had more severe hemolytic findings, larger sample sizes are needed to confirm this result.

Study Limitations

As a limitation of our study, functional research including animal experiments or cell cultures to evaluate the detected VUSs could not be performed. Therefore, further studies are necessary. In addition, this study was a single center study and more patients are needed to make clearer assessments of the genotype-phenotype correlations in HS patients.

Conclusion

This is the first cohort study in Türkiye on genotype-phenotype correlations in patients with HS. In addition, 9 novel variants were introduced to the literature. In our cohort, *SPTB* was the gene that had the most frequent variants and presented with a wide clinical spectrum. The patients with *ANK1* and *SPTA1* variants showed more severe clinical features, while patients with *EPB42* and *SLC4A1* variants were observed to have mild clinical findings. In the differential diagnosis of non-immune hemolytic anemias, non-specific parameters such as high MCHC are being replaced by NGS analysis.

Ethics

Ethics Committee Approval: The Local Ethics Committee of the Marmara University Faculty of Medicine approved the study (approval number: 1770, date: 08.12.2023).

Informed Consent: Written informed consent and/or assent was obtained from the children's parents or guardians.

Footnotes

Authorship Contributions

Surgical and Medical Practices: Ö.D., A.K.; Concept: Ö.D., A.K., P.A.; Design: Ö.D., A.K., P.A.; Data Collection or Processing: Ö.D., C.A.; Analysis or Interpretation: C.A., Ş.D., P.A.; Literature Search: Ö.D., C.A., Ş.D.; Writing: Ö.D., P.A.

Conflict of Interest: No conflict of interest was declared by the authors.

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